

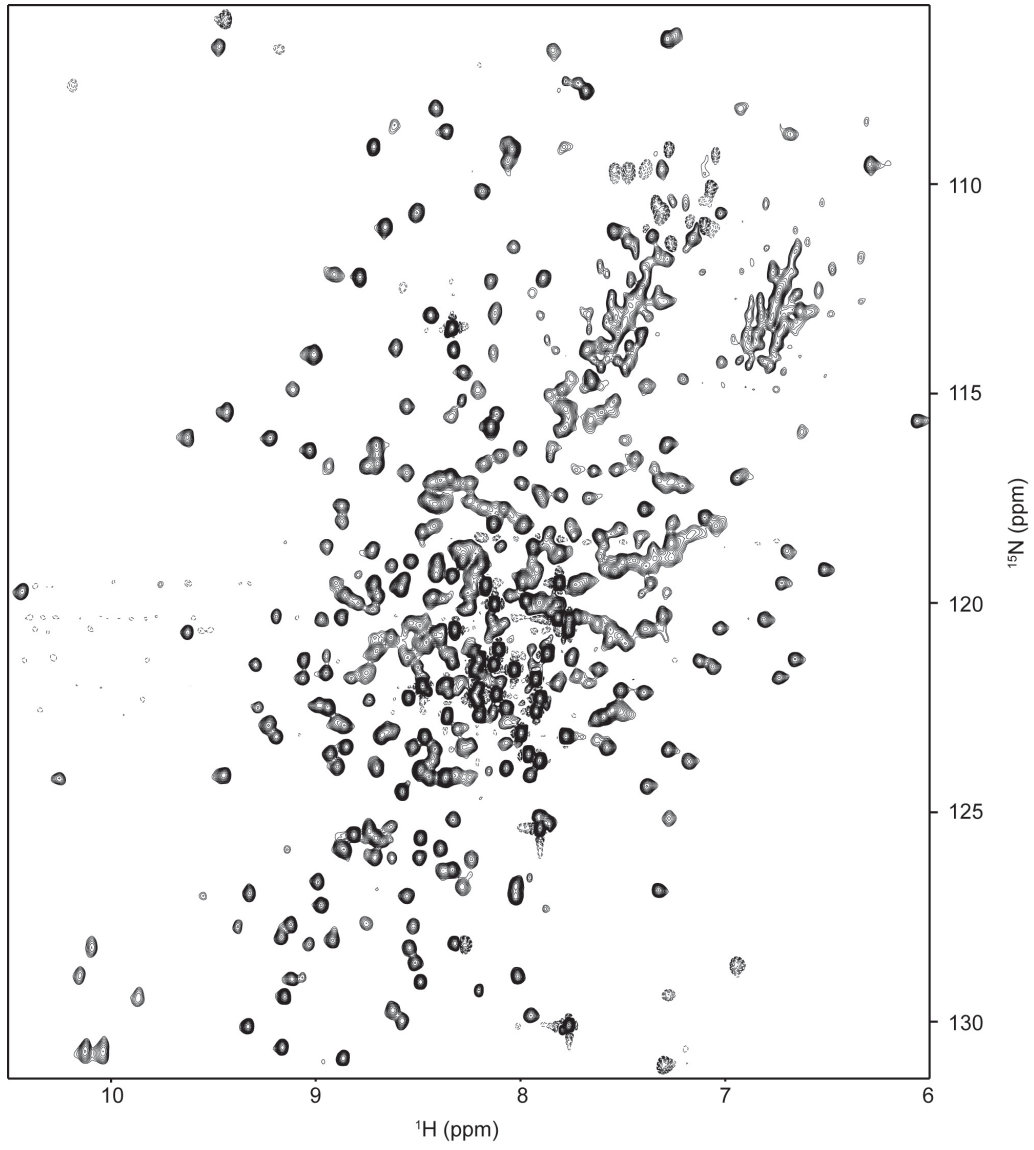
## Supplemental Information (Zwolak et al.)

### Figure Legends

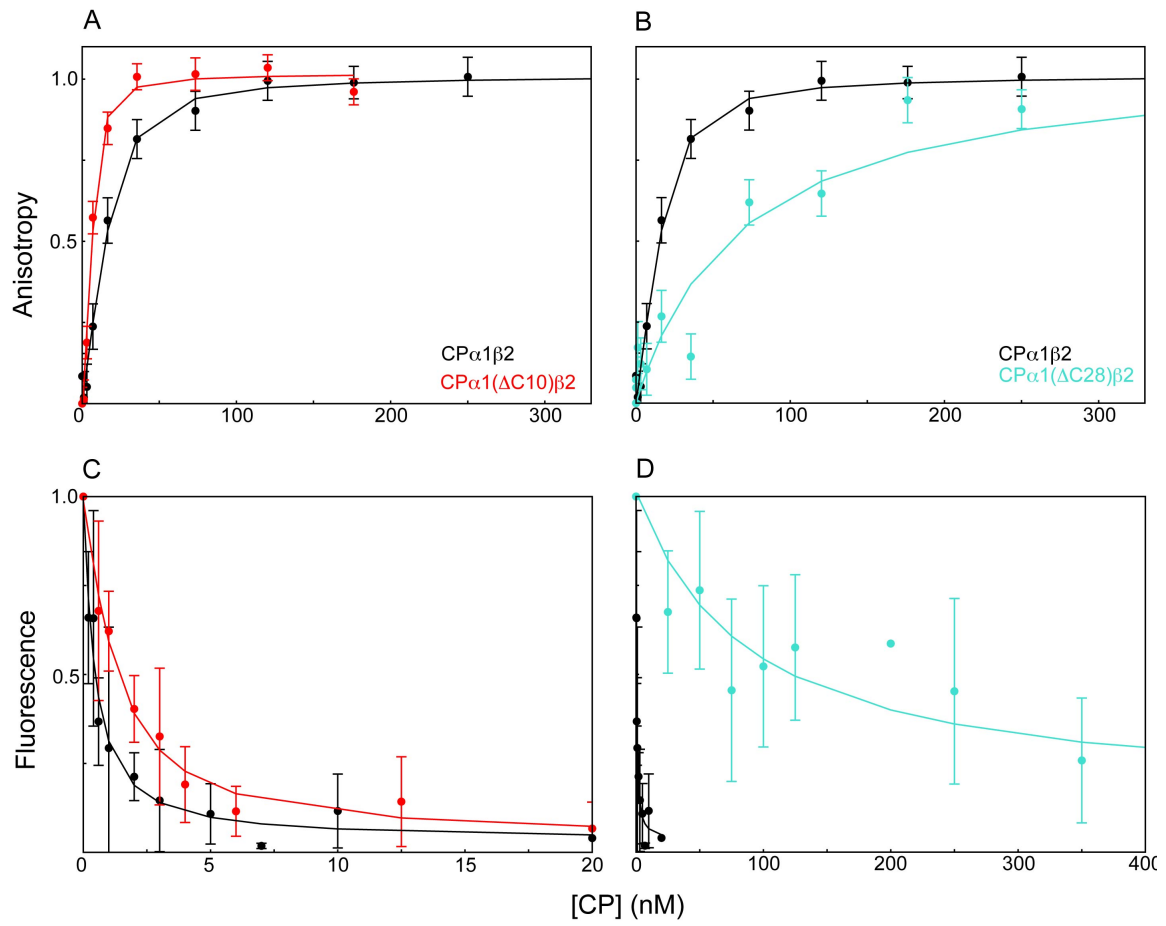
Supplemental Fig. 1. CP $\alpha$ 1 $\beta$ 2  $^{15}\text{N}$ - $^1\text{H}$ -TROSY HSQC spectrum. The HSQC spectrum of CP was recorded at 32 °C using an 800 MHz spectrometer equipped with a pulsed-field gradient cryogenically cooled probe using 32 transients with 1 s recycle delay between acquisitions, maximum acquisition of 61.4 ms in the indirect dimension.

Supplemental Fig. 2. CP $\alpha$  C-terminal deletion mutants elucidate the residues involved in V-1 and barbed end binding. Fluorescence anisotropy and pyrene-actin fluorescence measurements were performed as in Figure 8. Wild-type CP is shown in black in all panels. CP $\alpha$ 1( $\Delta$ C10) $\beta$ 2 (red) bound both V-1 (A) and barbed ends (C) with wild-type affinity while CP $\alpha$ 1( $\Delta$ C28) $\beta$ 2 (cyan) bound V-1 (B) and barbed ends (D) weakly (see Table 2 for numerical values).

Supplemental Figure 1



Supplemental Figure 2



**Supplemental Table 1. CP secondary shift average and secondary structure comparison to crystal structure**

chicken CP / mouse CP							
$\alpha 1/\alpha 1$				$\beta 1/\beta 2$			
2°	Residues	$\langle 2^\circ \text{ shift} \rangle$		2°	Residues	$\langle 2^\circ \text{ shift} \rangle$	
		$^{13}\text{C}^\alpha$	$^{13}\text{C}'$			$^{13}\text{C}^\alpha$	$^{13}\text{C}'$
H1	D10-T22	2.73	1.78	H1	D3-M13/L12	2.00	1.49
H2	F29-L40	2.63	1.58	H2	I21-L31	2.77	1.2
H3	D43-D60	2.14	1.62	H3	C36-S42	2.41	1.7
S1	T63-V65	-1.57	-1.27	S1	K48-D52	-1.68	-1.3
S2	V74-I76	-2.83	-2.37	S2	K57-L61	-1.56	-1.06
S3	R86-L88	-2.17	-1.23	S3	S70-R72	-1.37	-1.6
S4	I94-K97	-2.38	-1.33	H4	A91-F112	2.91	1.99
H4	K118-A135	2.48	1.99	S5	V-116-D123/W122	-1.33	-0.7
S5	G139-I148	-1.17	-1.39	S6	G127-A137	-1.32	-1.78
S6	Q151-Q164	-1.40	-1.56	S7	I144-E158	-1.38	-1.14
S7	W169-T180	-1.35	-0.70	S8	T164-K181	-1.36	-2.15
S8	T/S185-Y198	-1.10	-1.32	S9	G185/T186-	-1.04	-1.87
S9	V204-	-1.22	-1.67	H5	H209/N212-L243	2.89	1.42
H5a/H5	V/I222-D252	2.79	2.61	H6/-	Q/S253-T/K267	0.77	0.62
H5b/-	T254-L258	0.75	0.23				
H6	W271-I274	2.28	1.98				

**Supplemental Table 1.** Comparison of secondary structures of chicken CP $\alpha 1\beta 1$  to mouse CP $\alpha 1\beta 2$ . Chicken CP secondary structures are from the crystal structure. Mouse CP secondary structures were determined from NMR data. The  $\alpha$  subunits are compared on the left;  $\beta$  subunits, on right, with the chicken naming being the same as in the mouse, except as indicated by a “/” where the mouse sequence or structure is different from that of chicken. Secondary structures which could not be